Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes

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Abstract

The effect of cream pasteurization on the composition and microstructure of buttermilk after pasteurization, evaporation and spray-drying was studied. The composition of milk fat globule membrane (MFGM) isolated from buttermilk samples was also characterized. Pasteurization of cream resulted in higher lipid recovery in the buttermilk. Spray-drying of buttermilk had a significant effect on phospholipid content and composition. After spray-drying, the phospholipid content decreased by 38.2% and 40.6%, respectively in buttermilk from raw or pasteurized cream when compared with initial buttermilks. Pasteurization of cream resulted in the highest increase in whey protein recovery in MFGM isolates compared with all other processing steps applied on buttermilk. A reduction in phospholipid content was also observed in MFGM isolates following spray-drying. Transmission electron microscopy of the microstructure of buttermilks revealed extremely heterogeneous microstructures but failed to reveal any effect of the treatments.

Keywords: Buttermilk; Milk fat globule membrane; Heat treatments; Phospholipids; Cream

1. Introduction

Buttermilk is the liquid phase released during churning of cream in the process of butter making. This liquid phase contains most of the water-soluble components of cream. After disruption of fat globules, milk proteins, lactose, minerals and some lipids are recovered in buttermilk as well as milk fat globule membrane (MFGM) fragments. MFGM is composed mainly of proteins, phospholipids and minerals (Walstra, Wouters, & Geurts, 2006). The MFGM fragments are of particular importance considering the various health-related properties described for its components (Spitsberg, 2005). For example, it was reported recently that MFGM fractions obtained from buttermilk and whey buttermilk have an anti-viral effect on rotaviruses strains (Ochonicky, Donovan, Kuhlenschmidt, Jiménez-Flores, & Kuhlenschmidt, 2005). Also, phospholipids have been reported as having potential physiological effects on brain health (Kidd, 2000), cholesterol binding in vivo (Noh & Koo, 2004), stress management (Rutenberg, 2002) and inhibition of tumour growth (Schmelz, Sullards, Dillehay, & Merrill, 2000).

Industrial treatments are known to have a major impact on the MFGM (van Boekel & Walstra, 1995). Heat is arguably the single most important factor affecting the MFGM. Adsorption of copper from milk plasma, aggregation of MFGM proteins, loss of MFGM proteins and phospholipids, and adsorption of caseins and whey proteins on the surface of the MFGM have been reported (Evers, 2004). Interactions between whey proteins and the MFGM also have been reported and are believed to be partly caused by sulphydryl–disulphide interactions (Dalgleish & Banks, 1991; Kim & Jiménez-Flores, 1995; Lee & Sherbon, 2002; Ye, Singh, Taylor, & Anema, 2002). Houlihan, Goddard, Kitchen, and Masters (1992) found both β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) bound to MFGM after heating whole milk at 80 °C. Ye, Singh, James, and Anema (2004) proposed that interactions between β-LG and MFGM proteins were temperature dependent. Thermal denaturation of both β-LG and MFGM proteins results in disulphide linkage of MFGM

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aggregates and β-LG complexes. In another study, Lee & Sherbon (2002) reported that MFGM in milk heated for 3 min at 80 °C contain approximately 0.03 g of β-LG and 0.008 g of α-LA per 100 g of fat globules. These amounts increased with increased heating time. Lee and Sherbon (2002) also reported a loss of about 20% of lipids when MFGM are heated. However, the authors did not report the composition of the lipids lost during the heating process. It is believed that the migration of lipids from MFGM to the serum only occurs in presence of serum components (Houlihan et al., 1992).

To date, all the data collected on the effects of heat treatments on MFGM have been obtained after heat treatment of whole raw milk, which implies that the milk fat globules were structurally intact during heating. However, in the case of buttermilk, MFGM are not globular but rather sheet-like (Corredig & Dalgleish, 1997). MFGM components in both the inner and outer layers of the membrane are exposed to buttermilk serum. The interactions or repulsions between components might be drastically different from that of whole milk fat globules. At the industrial scale, buttermilk is often subjected to severe conditions (long holding time before evaporation and spray-drying, higher pasteurization temperatures for sanitary reasons), which are likely to induce changes in buttermilk microstructure.

Buttermilk can be a concentrated source of MFGM components. A number of studies aimed at concentrating or isolating MFGM from buttermilk have been reported (Corredig, Roesch, & Dalgleish, 2003; Jiménez-Flores & Morin, 2005; Morin, Jiménez-Flores, & Pouliot, 2004; Sachdeva & Buchheim, 1997; Surel & Famelart, 1995). Major variations in fractionation yields have been observed with buttermilks that had been obtained from different sources or had undergone different industrial treatments (Astaire, Ward, German, & Jiménez-Flores, 2003; Morin et al., 2004). Morin et al. (2004) used microfiltration (MF) membranes to separate phospholipids from MFGM and showed that the passage of phospholipids through the MF membranes was greatly affected by filtration temperature. In the same study, using the same filtration procedure, the authors found that phospholipid transmission was reduced by 50% when fresh pasteurized buttermilk was used as opposed to reconstituted powdered buttermilk (Morin et al., 2004). The major difference between the products was the processing history, indicating that processing steps may have a major impact on buttermilk phospholipids and MFGM. Little is known about the composition and microstructure of buttermilk and MFGM, which might explain the limited success of MFGM fractionation by MF.

Understanding the changes that occur in buttermilk composition and microstructure as a function of processing history could help to improve MF fractionation and enhance our understanding of the properties of buttermilk as a functional ingredient. The present study was aimed at investigating the effects of cream pasteurization and of the buttermilk processing steps, namely pasteurization, evaporation and spray-drying, on buttermilk composition and microstructure as well as on the composition of MFGM isolated from buttermilk.

2. Materials and methods

2.1. Processing conditions

Fresh raw cream (110 L; 43–44% fat) separated from raw milk at 35 °C was obtained from a local dairy (Natrel, Québec City, Canada) and divided into two batches on reception. One batch was pasteurized at 85 °C for 20 s using a tubular pasteurizer (Actini, Model Mini-Actijoule, Evian-Les-Bains, France). The pasteurized cream and raw cream were then stored at 10 °C overnight for maturation. Both cream samples were subsequently churned in a rotary churn (Fromagex, Rimouski, Canada) at 26 rpm and 13 °C. The raw and pasteurized creams broke down within an average ($n = 2$) of 23.3 ± 0.3 and 27 ± 0.8 min, respectively. The buttermilk was recovered in milk cans after separating the butter fines using a stainless steel filter. Residual lipids from both buttermilks types were removed by centrifugation using a milk separator (DeLaval model No. 619, Lund, Sweden) running at 6000 rpm and 18 °C. Sodium azide (0.02% (w/v)) (Fisher Scientific, Nepean, ON, Canada) was added as a preservative and samples were withdrawn for analysis. Both buttermilk types were pasteurized at 72 °C for 20 s using the tubular pasteurizer and samples were collected. Before evaporation, 30 ppm of anti-foaming agent (Dow Corning, Varennes, QC, Canada) was added. Each pasteurized buttermilks was then evaporated using a falling-flow evaporator (Mojonnier, LTS1 Laboratory Model Lo-Temp Evaporator, Chicago, IL, USA) at 60 °C until 20% solids was reached as measured using a handheld refractometer. A sample of concentrated buttermilk was collected for analysis. Lastly, the concentrated buttermilk was spray-dried using a pilot plant spray-dryer (Niro A/S, Hudson, WI, USA). Inlet and outlet air temperature were set at 195 and 85 °C, respectively. Samples of the powders were collected for analysis. The entire process has been repeated twice.

2.2. Isolation of MFGM

A sample of buttermilk (350 mL) obtained after each processing step was used for MFGM isolation with a slightly modified version of the procedure of Corredig and Dalgleish (1997). Briefly, sodium citrate (2% w/v) was added to buttermilks from raw and pasteurized creams. Buttermilk was stored at 4 °C overnight for maximum micelle dissociation. They were then centrifuged at $50{,}000 \times g$ at 4 °C for 2 h. The pellets were collected on Whatman #1 filter paper and rinsed with 25 mL of deionized water. The pellets were re-suspended in 100 mL of deionized water using a benchtop homogenizer (Polytron PT 3100, Brinkman, Westbury,

NY, USA). The suspensions were subsequently freeze-dried and stored at −20 °C. The same procedure was followed for concentrated samples, except that the samples were suspended to 10% total solids using deionized water and were equilibrated overnight at 4 °C before isolating the MFGM.

2.3. Analytical procedures

2.3.1. Compositional analyses

Buttermilks and MFGM isolates were analyzed for total protein using a nitrogen analyzer (Leco FP-528, Leco Corp., St. Joseph, MI, USA) and a protein conversion factor of 6.38. Total solids were obtained by microwave dying (Smart System 5, CEM Corp., Matthews, NC, USA) and ash was measured by incineration in a muffle furnace at 550 °C for 20 h. Lipids were extracted using the Mojonnier ether extraction procedure, and lipid extracts were diluted to 5 mg mL^{-1} in 2:1 chloroform:methanol and stored at −20 °C until further analysis. The lipid profiles of the buttermilks were obtained by HPLC-ELSD as described previously (Morin et al., 2004). MFGM isolate protein profiles were obtained by SDS-PAGE as described previously (Morin, Pouliot, & Jiménez-Flores, 2006) and protein class distribution was estimated by densitometry (Gel Doc XR, Bio-Rad, Hercules, CA, USA). Every analysis was performed at least in duplicate and all reagents were HPLC grade.

2.3.2. Transmission electron microscopy (TEM)

The structure of buttermilk samples was observed by TEM using the procedure described by Corredig and Dalgleish (1997). Briefly, samples of buttermilk were centrifuged at 15 °C at 50,000 × *g* for 2 h. The pellets were recovered and fixed in glutaraldehyde and post-fixed in osmium tetraoxide. Samples were then dehydrated in a series of graded ethanol baths and embedded in epoxy resin. Thin sections were stained using lead citrate. The transmission electron microscope (JEOL 1230, JEOL products, Peabody, MA, USA) was used at 80 KV. Micrographs shown are representative of views of two different observation grids for each sample of each replicate.

2.4. Statistical analysis

Statistical analyses were performed using Minitab 14.0 software (State College, PA, USA). ANOVA with Tuckey's comparison test was carried out on the means. Differences were considered statistically significant at $p<0.05$.

3. Results and discussion

3.1. Effect of pre-churning pasteurization of the cream on the composition of buttermilk

The composition of the different buttermilks is reported in Table 1. The protein, lipid and ash contents of the buttermilks were in the ranges of previously reported data (Astaire et al., 2003; Mistry, Metzger, & Maubois, 1996). However, the total lipid content of buttermilk from pasteurized cream was higher than reported values. The main compositional difference observed between buttermilks from raw and pasteurized cream was the total fat content. Buttermilk from pasteurized cream contained twice as much fat as buttermilk from raw cream. Given the fact that the same skimming conditions were used for both creams, the higher fat content in the buttermilk from pasteurized cream was somewhat unexpected. This observation may be related to the fact that pasteurization of cream induces the deposition of caseins and whey proteins at the surface of fat globules and therefore limits the coalescence of the globules (Dalgleish & Banks, 1991; Houlihan, et al., 1992; Ye, Anema, & Singh, 2004). This deposition might be induced either by the heat treatment itself or by partial homogenization in the pasteurization equipment. This explanation is supported by the longer churning time required with pasteurized cream (27 vs. 23 min). Small, undisrupted fat globules would therefore be recovered in the buttermilk. It is also known that pasteurization of cream at temperatures higher than 78 °C leads to complete inactivation of agglutinins (Walstra et al., 2006). Therefore, the high fat content in the

Table 1
Composition of buttermilk after each processing step

Buttermilk processing step	% Dry matter[a]					
	Proteins		Lipids		Ash	
	RCB[b]	PCB	RCB	PCB	RCB	PCB
Separation	36.5^{a,y}	31.5^{a,z}	8.09^{a,y}	20.2^{a,z}	7.32^{a,y}	6.33^{a,y}
Pasteurization	36.1^{a,y}	31.8^{a,z}	7.86^{a,y}	19.5^{a,z}	7.36^{a,y}	6.49^{a,y}
Evaporation	35.1^{a,y}	30.2^{a,z}	8.11^{a,y}	19.4^{a,z}	7.43^{a,y}	6.33^{a,z}
Spray-drying	35.4^{a,y}	31.1^{a,z}	8.29^{a,y}	19.7^{a,z}	7.74^{a,y}	6.76^{a,z}

[a]Means ($n = 2$) with different superscript letters (a,b) in the same sub-column (RCB or PCB) differ significantly ($p<0.05$). Means ($n = 2$) with different superscript letters (y,z) for the same processing step for each components (proteins, lipids or ash) differ significantly ($p<0.05$).
[b]RCB, raw cream buttermilk; PCB, pasteurized cream (85°C/20 s) buttermilk.

Table 2
Phospholipid composition of buttermilk after each processing step

Buttermilk processing step	% Total phospholipids[a]											
	Total phospholipids (% dry matter)[b]		PE		PC		SM		PS		PI	
	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB
Separation	$1.49^{a,y}$	$1.28^{a,z}$	$51.2^{a,y}$	$43.2^{a,y}$	$23.0^{a,y}$	$23.2^{a,y}$	$22.2^{a,y}$	$29.7^{a,z}$	$2.97^{a,y}$	$1.48^{a,y}$	$0.67^{a,y}$	$1.13^{a,y}$
Pasteurization	$1.42^{a,y}$	$1.19^{a,y}$	$50.6^{a,y}$	$41.7^{a,y}$	$22.5^{a,y}$	$23.8^{a,y}$	$23.1^{a,y}$	$30.7^{a,z}$	$3.08^{a,y}$	$1.32^{a,y}$	$0.79^{a,y}$	$1.31^{a,y}$
Evaporation	$1.54^{a,y}$	$1.18^{a,y}$	$48.4^{a,y}$	$42.2^{a,y}$	$23.3^{a,y}$	$23.3^{a,y}$	$23.4^{a,y}$	$30.4^{a,z}$	$3.90^{a,y}$	$1.37^{a,y}$	$1.08^{a,y}$	$1.32^{a,y}$
Spray-drying	$0.92^{b,y}$	$0.76^{b,y}$	$29.4^{b,y}$	$24.9^{a,y}$	$34.4^{b,y}$	$33.9^{a,y}$	$34.2^{a,y}$	$41.1^{b,z}$	$1.73^{a,y}$	$1.04^{a,y}$	$0.32^{a,y}$	$0.97^{a,y}$

[a]Means ($n = 2$) with different superscript letters (a,b) in the same sub-column (RCB or PCB) differ significantly ($p < 0.05$). Means ($n = 2$) with different superscript letters (y,z) for the same processing step for each components (Total phospholipids, PE, PC, SM, PS or PI) differ significantly ($p < 0.05$).
[b]PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; PS, phosphatidylserine; PI, phosphatidylinositol; RCB, raw cream buttermilk; PCB, Pasteurized cream (85 °C/20 s) buttermilk.

pasteurized cream buttermilk is likely to be more difficult to separate from skimmed buttermilk considering that inactivated agglutinin cannot induce creaming. Also, instability of raw cream could partly explain the lower fat content of raw cream buttermilk since active enzymes and lipases could have damaged part of the MFGM in raw cream before churning. This possibly allowed for faster churning of raw cream and better recovery of lipids in the butter therefore inducing lower loss of lipids in the unskimmed buttermilk. Pasteurization resulted in significantly lower amounts of proteins ($p < 0.05$), ash ($p < 0.05$) (Table 1) and phospholipids ($p < 0.05$) (Table 2) in buttermilk. These lower values resulted from the higher total fat content of the pasteurized cream buttermilk since all the data are reported as a proportion of total solids. Indeed, no significant differences were observed among samples when the data were compared on a wet basis (not shown).

3.2. *Effect of post-churning processing steps on the composition of buttermilk*

The compositional data reported in Table 1 provide evidence that the effect of the processing steps on the composition of buttermilk was minimal for total proteins, lipids and ash. Total solid levels of 40–50% after evaporation are typically obtained in dairy processes but excessive foaming in spite of added anti-foam was observed in the evaporation equipment so the process had to be stopped at 20% total solids. The phospholipid content and profile of the buttermilks at each processing step are shown in Table 2. The phospholipid content decreased significantly for both buttermilks after spray-drying. In the case of raw cream buttermilk the relative decrease compared to skimmed buttermilk was 38.2% (from 1.49% to 0.92%) and 40.6% (from 1.28% to 0.76%) for pasteurized cream buttermilk. Most phospholipids in buttermilk are contained in MFGM fragments. A loss of all major phospholipids would thus be an indication of a loss of whole MFGM fragments. We observed that the proportion of phosphatidylethanolamine (PE) decreased during spray-drying. Phosphatidylserine (PS) and phosphatidylinositol (PI) also decreased somewhat but to a much smaller extent while the relative distribution of phosphatidylcholine (PC) and sphingomyelin (SM) increased. The decrease in phospholipid content seemed to be attributable mainly to PE since PS and PI accounted for only a very small proportion of total buttermilk phospholipids.

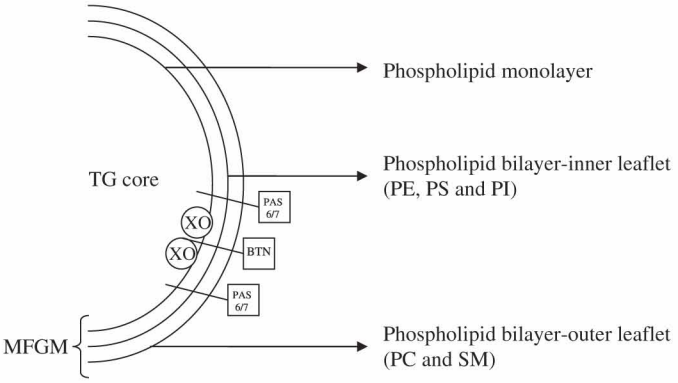

Fig. 1. Schematic representation of MFGM (adapted from Danthine et al., 2000). Lipids: TG, tryglycerides; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin. Proteins: XO, xanthine oxidase; PAS 6/7, periodic acid shift 6/7; BTN, butyrophilin.

According to the most recent structural model of MFGM, the three phospholipids that decreased in our trials are located in the inner leaflet of the membrane and are in contact with the monolayer of phospholipids surrounding the triglyceride core (Fig. 1) (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000). In milk, this portion of the membrane is buried inside the fat globule, thus preventing interactions with serum proteins and caseins. However, in the case of buttermilk, the inner leaflet of the membrane is exposed to the serum and processing can induce interactions and possible dislocation of the membrane. The fate of the phospholipids lost from buttermilk after spray-drying is unclear. The observed decrease suggests that processing modified the aggregation of the phospholipids, resulting in decreased solubility in the extraction solvents. It has been reported that MFGM

proteins are very reactive at relatively low temperatures (60 °C) (Ye et al., 2002) and that heating at 80 °C causes a total loss of MFGM protein Periodic acid Schiff 6/7 (PAS 6/7) from MFGM (Lee & Sherbon, 2002). In human milk fat, PAS 6/7 has been identified as lactadherin and is believed to be involved in phospholipid binding (Fortunato et al., 2003). Heat treatment combined with the rapid water removal and increased ionic strength created by spray-drying could induce the formation of complexes among milk proteins, MFGM proteins and phospholipids, rendering the phospholipids non-extractable by the Mojonnier procedure, thus causing an apparent decrease in phospholipids. Whether the observed decrease in phospholipid content is caused by interactions of specific phospholipids with serum material or with MFGM fragments that render extraction more difficult requires further investigation. Phospholipid extraction using ether, as with the Mojonnier method, is a standard method for dairy products and has been used in other buttermilk fractionation studies (Astaire et al., 2003; Jiménez-Flores & Morin, 2005; Morin et al., 2004; Sachdeva & Buchheim, 1997). However, in light of our results, special attention should be paid to lipid extraction procedures in order to verify the effectiveness of the method used for extracting buttermilk phospholipids after the different processing steps.

3.3. *Effect of pre churning cream pasteurization on the composition of MFGM isolates*

The compositions of the MFGM isolates, the protein and phospholipid profiles of the isolates are shown in Tables 3, 4 and 5, respectively. Pasteurization of the cream significantly increased ($p<0.05$) protein recovery in the MFGM isolates from buttermilk. Protein profiling by SDS-PAGE (Fig. 2 and Table 4) showed that whey proteins in the MFGM isolates accounted for this increase in protein content. Pasteurization of the cream resulted in an almost 3-fold increase in the amount of whey proteins bound to the MFGM in the resulting buttermilk compared to buttermilk produced from raw cream (20.4 vs. 7.34%). The heat treatment of the cream resulted in high incorporation of β-LG in the MFGM isolate (Fig. 2, lanes 1 vs. 5) and β-LG appears to be the main protein in the MFGM isolates from pasteurized cream as judged by the staining intensity in the SDS-PAGE gel. This result is in accordance with data reported by Corredig and Dalgleish (1998). This suggests that any approach used to isolate or concentrate MFGM by MF or other physical separation techniques using pasteurized cream buttermilk as a substrate will necessarily yield an MFGM isolate or concentrate with a high concentration of associated β-LG. The interaction of β-LG with MFGM proteins when heated has been previously reported (Kim & Jiménez-Flores, 1995; Ye, Singh, James, et al., 2004; Ye et al., 2002; Ye, Singh, Taylor, et al., 2004), and is mainly caused by disulphide bonding. Heat treatment of the cream also results in significantly less ash being recovered in the MFGM isolates from pasteurized buttermilk ($p<0.05$) (Table 3). MFGM powder from raw cream is reddish-brown as compared with yellow-white from pasteurized cream. This has also been reported by Corredig and Dalgleish (1998) and is believed to be caused by a decrease in the amount of iron in the MFGM, which may also be partly responsible for the difference in the amount of total ash.

3.4. *Effect of post churning buttermilk processing steps on the composition of MFGM isolates*

The processing steps of buttermilk resulted in no significant changes in the total protein, lipid and ash composition of the MFGM isolates (Table 3). However, there was a significant increase ($p<0.05$) in whey protein content in the MFGM isolated from raw cream buttermilk (Table 4) and the amount never reached that in the MFGM isolated from pasteurized cream. The intensity of the heat treatment applied to the cream was more important (85 °C/20 s) than any of the subsequent processing steps. Pasteurization of the cream may therefore result in more interactions between MFGM and whey proteins. This result is in accordance with the findings of Corredig and Dalgleish (1998), who reported that heat treatment of cream is a critical step in determining the composition and functional properties of MFGM proteins recovered from

Table 3
Composition of MFGM isolates from buttermilk

Buttermilk processing step	% Dry matter[a]					
	Proteins		Lipids		Ash	
	RCB[b]	PCB	RCB	PCB	RCB	PCB
Separation	56.2^{a,y}	69.5^{a,z}	18.9^{a,y}	13.6^{a,y}	5.99^{a,y}	4.79^{a,y}
Pasteurization	61.1^{a,y}	70.0^{a,z}	18.8^{a,y}	13.8^{a,y}	5.41^{a,y}	4.69^{a,z}
Evaporation	60.7^{a,y}	69.2^{a,y}	16.5^{a,y}	15.3^{a,y}	6.02^{a,y}	4.80^{a,y}
Spray-drying	63.0^{a,y}	65.4^{a,y}	13.6^{a,y}	14.1^{a,y}	5.24^{a,y}	4.90^{a,y}

[a]Means ($n = 2$) with different superscript letters (a,b) in the same sub-column (RCB or PCB) differ significantly ($p<0.05$). Means ($n = 2$) with different superscript letters (y,z) for the same processing step for each components (proteins, lipids or ash) differ significantly ($p<0.05$).
[b]RCB, raw cream buttermilk; PC, pasteurized cream (85 °C/20 s) buttermilk.

Table 4
Protein composition of MFGM isolates from buttermilk

Buttermilk processing step	% Protein in dry matter[a]					
	Casein		Whey		MFGM	
	RCB[b]	PCB	RCB	PCB	RCB	PCB
Separation	9.54^{a,y}	13.6^{a,y}	7.35^{a,y}	20.4^{a,z}	25.3^{a,y}	23.5^{a,y}
Pasteurization	12.8^{a,y}	13.3^{a,y}	13.7^{b,c,y}	20.5^{a,y}	23.3^{a,y}	24.1^{a,y}
Evaporation	13.8^{a,y}	14.2^{a,y}	12.7^{b,y}	19.6^{a,z}	22.2^{a,y}	22.2^{a,y}
Spray-drying	13.5^{a,y}	13.4^{a,y}	15.6^{c,y}	20.1^{a,y}	21.2^{a,y}	21.6^{a,y}

[a]Protein (casein, whey and MFGM) distribution estimated by Coomassie blue staining intensity in SDS-PAGE gels. Means ($n = 2$) with different superscript letters (a,b,c) in the same sub-column (RCB or PCB) differ significantly ($p < 0.05$). Means ($n = 2$) with different superscript letters (y,z) for the same processing step for each protein class (caseins, whey or MFGM) differ significantly ($p < 0.05$).
[b]RCB, raw cream buttermilk; PCB, pasteurized cream (85°C/20 s) buttermilk.

Table 5
Phospholipid composition of MFGM isolates from buttermilk

Buttermilk processing step	% Total phospholipids[a]											
	Total phospholipids (% dry matter)[b]		PE		PC		SM		PS		PI	
	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB	RCB	PCB
Separation	6.35^{a,y}	6.32^{a,y}	34.0^{a,y}	33.1^{a,y}	28.8^{a,y}	30.5^{a,y}	34.6^{a,y}	35.2^{a,y}	1.47^{a,y}	0.00^{*a,y}	1.12^{a,y}	0.44^{a,y}
Pasteurization	6.59^{a,y}	6.24^{a,y}	38.6^{a,y}	38.5^{a,y}	27.0^{a,y}	28.9^{a,y}	31.8^{a,y}	30.5^{a,y}	1.32^{a,y}	0.80^{a,y}	1.31^{a,y}	0.44^{a,y}
Evaporation	6.33^{a,y}	5.78^{ab,y}	36.7^{a,y}	41.2^{a,y}	27.8^{a,y}	26.8^{a,y}	32.8^{a,y}	30.8^{a,y}	1.36^{a,y}	0.00^{a,y}	1.31^{a,y}	0.42^{a,y}
Spray-drying	4.75^{a,y}	4.43^{b,y}	26.8^{a,y}	29.5^{a,y}	35.0^{b,y}	34.0^{a,y}	36.2^{a,y}	35.5^{a,y}	1.04^{a,y}	0.00^{a,y}	0.97^{a,y}	0.38^{a,y}

[a]Means ($n = 2$) with different letters (a,b) in the same sub-column (RCB or PCB) differ significantly ($p < 0.05$). Means ($n = 2$) with different letters (y,z) for the same processing step for each components (Total phospholipids, PE,PC,SM, PS or PI) differ significantly ($p < 0.05$). 0.00, PS peak not detected by HPLC-ELSD.
[b]PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; RCB, raw cream buttermilk; PCB, Pasteurized cream (85 °C/20 s) buttermilk.

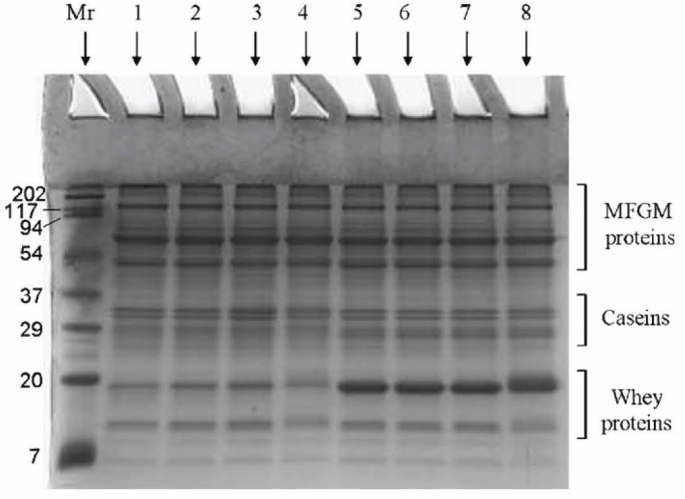

Fig. 2. SDS-PAGE of MFGM isolates from raw cream buttermilk after separation (1), pasteurization (2), evaporation (3) and spray-drying (4) and pasteurized cream buttermilk after separation (5), pasteurization (6), evaporation (7) and spray-drying (8). Mr, molecular mass standard (kDa).

buttermilk. Results also showed a slight decrease in the MFGM protein content of the isolates from both buttermilks. This decrease was not statistically significant given that only two replicates were performed and that the quantification was based on densitometric analyses of SDS-PAGE gels. The MFGM proteins may have been displaced from the membrane by skim milk proteins and may therefore not have been recovered in the MFGM isolates.

A high proportion of caseins was recovered in the MFGM isolates, accounting for up to 14.2% of the dry matter in evaporated buttermilk from pasteurized cream (Table 4). The origin of these caseins may have been incidental (a result of the isolation procedure) or caused truly by processing. The method used for MFGM isolation employs centrifugation to recover the MFGM. Citrate (2%), which acts as a calcium chelating agent, is added to break down the casein micelle structure in order to retain the caseins in the serum (Corredig & Dalgleish, 1998). However, MFGM may capture and drag some of the casein with them into the pellet. The high proportion of casein in the MFGM fraction suggests that the same problem may occur during membrane filtration of buttermilk and thus explain the poor separation between MFGM and caseins obtained in MF of buttermilk (Morin, et al., 2004).

It is well known that the homogenization of milk results in the incorporation of casein micelles in MFGM (Walstra,

Wouters, & Geurts, 2006). During churning of the cream, partial homogenization may occur, causing caseins to interact with MFGM and to be recovered in the MFGM fraction. The casein present in MFGM isolated from whole milk is mostly the result of mechanical treatments rather than heating (Dalgleish & Banks, 1991). This might also be the case for buttermilk. Even after extensive washing, skim milk proteins like β-casein and α-LA can still be detected in rather large proportions (13%) in human fat globules by a proteomic approach using two-dimensional gel electrophoresis (Fortunato et al., 2003). This further supports the fact that skim milk proteins may interact strongly with MFGM even before milk is collected.

A decrease in the total phospholipid content of MFGM isolates also occurred (Table 5) after spray-drying. This was mostly characterized by a decrease in PE, which was also observed in the buttermilks. It is thus likely that the decrease was mainly caused by changes in the MFGM fragments rather than in free phospholipids in the buttermilk. Conflicting results on the effect of heating on phospholipids from whole milk fat globules have been reported (Evers, 2004). No data are available to compare

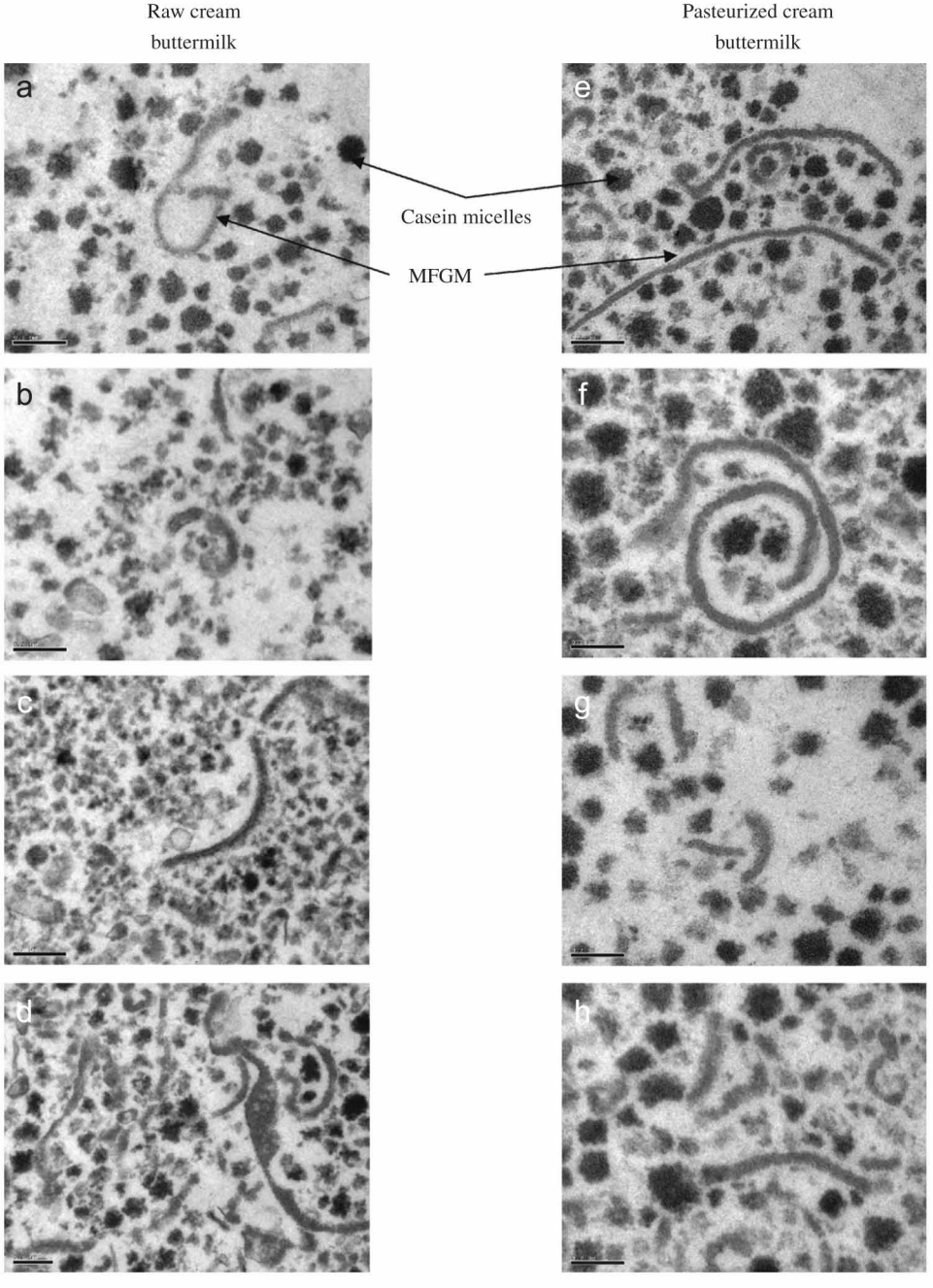

Fig. 3. Transmission electron micrographs of initial buttermilk (a,e), pasteurized buttermilk (b,f), evaporated buttermilk (c,g) and spray-dried buttermilk (d,h). Bars = 0.2 μm.

the effect of heating on phospholipids in ruptured milk fat globules.

3.5. *Effect of processing on the microstructure of buttermilk*

In an attempt to show a relationship between the observed compositional changes in buttermilk with changes in buttermilk microstructure, TEM was used to examine buttermilk samples after the different processing steps. TEM micrographs are shown in Fig. 3. The size of the MFGM fragments was highly variable, ranging from 0.1 to 2–3 μm, and should be related to the size of the initial fat globule before churning as well as to the strength of the bonds between the MFGM molecules. Membrane fragments were also found to be flexible, as a large proportion of the fragments appeared folded and deformed. Based solely on the TEM observations, it was impossible to determine whether the heat treatment of the cream or the processing steps to produce the buttermilk had any effect on the microstructure of the MFGM fragments. However, some rather intriguing observations were made. The TEM micrographs show that MFGM fragments can fold and trap casein micelles inside a reconstituted globule (Figs. 3d–f). Even tough the caseins appear to be simply physically trapped by the MFGM fragments, this observation indicates that MFGM may interact with casein micelles. In the context of buttermilk fractionation, these interactions are important for concentrating MFGM and for buttermilk functional properties. The poor separation of MFGM and casein micelles during MF of buttermilk may be partially due to such interactions. Moreover, several studies have reported that buttermilk performs poorly during rennet coagulation for cheese making (Sachdeva & Buchheim, 1997; Turcot, Turgeon, & St-Gelais, 2001). The interactions of MFGM fragments with casein micelles might prevent gel formation and create a weak coagulum. The observed loss of phospholipids after spray-drying of the buttermilks could not be correlated with the TEM observations of the buttermilks. Either the loss of phospholipids did not affect the structure of the MFGM fragments or the changes were too subtle to be clearly seen in the TEM micrographs since the phospholipids may simply have become unextractable.

4. Conclusion

Pasteurization of cream appeared to be the critical step in the modification of MFGM composition, especially in terms of total lipid content. Spray-drying seemed to have a strong influence on the amount and distribution of phospholipids recovered in buttermilk. The exact nature of the observed changes in phospholipid content is not known but might reflect modifications in the structure of MFGM. Modifications in MFGM structure were not found based upon direct TEM observations of buttermilk. The present study provides evidence that the processing of buttermilk can cause major changes in both buttermilk and MFGM fragments. The TEM micrographs support the view that the heterogeneous microstructure of buttermilk explains the unsatisfactory results of MF fractionation of buttermilk.

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